CHROMATOPHOROTROPINS IN THE CENTRAL NERVOUS ORGANS OF THE CRAB, HEMIGRAPSUS OREGONENSIS

THOMAS E. BOWMAN [1]

Department of Zoology, University of California, Berkeley 4

Introduction

Early work on the humoral control of crustacean chromatophores has demonstrated that the sinus gland is the most important source of chromatophorotropic substances. Investigations leading to this conclusion are discussed in Brown's review (1944, pp. 130–134). Later work by Brown (1946) and Brown and Saigh (1946) has shown that most crustacean central nervous systems also possess at least two chromatophorotropic principles, one causing all portions of the body of Crago except the telson and uropods to become pale (Crago body-lightening hormone, CBLH), and a second (Crago-darkening hormone, CDH) darkening the telson and uropods, and in the absence of CBLH, the body also. CDH, however, was absent in the Brachyura studied.

This paper reports experiments undertaken for the purpose of determining whether or not chromatophorotropins are present in the central nervous system of the Pacific coast shore crab, *Hemigrapsus oregonensis*. Most attention was given to the optic ganglia, but some experiments were performed to test the brain and thoracic ganglia. I wish to thank Dr. R. I. Smith for his many helpful suggestions and criticisms.

Materials and Methods

Only male crabs were used. Their eyestalks were ligated with number 80 cotton thread on successive days before injections were made. After the melanophores were completely punctate, the crabs were injected with Carcinides perfusion fluid (Pantin, 1934), and those whose melanophores responded at all were not used in subsequent experiments.

Organs from which extracts were made were rinsed in several changes of Carcinides perfusion fluid to remove any blood adhering to them and transferred to a roughened depression slide containing a drop or two taken from a measured quantity of perfusion fluid. Here they were torn apart, crushed, and triturated with fine forceps under a dissecting microscope, care being taken to ensure as complete extraction as possible. The extract was then transferred with an eyedropper to the measured quantity of perfusion fluid, and the depression slide was rinsed with this perfusion fluid several times. The extract was boiled for a few seconds, allowed to settle, and the supernatant fluid was used for injections. Sterile needles and syringes were used for all injections.

[1] Present address: Scripps Institution of Oceanography, La Jolla, California. The material reported herein is from a thesis submitted to the Faculty of the University of California in partial fulfillment of the requirements for the degree of Master of Arts.

To record the responses of the melanophores, an arbitrary index of four stages was used, from complete concentration of the pigment (stage 1) to complete dispersion (stage 4). The somewhat opaque and pigmented cuticle of *Hemigrapsus oregonensis,* especially in the larger crabs, obscures the chromatophores over much of the body, and observations were therefore made on the arthrodial membranes at the bases of the legs.

In preliminary experiments it was found that the melanophores of *H. oregonensis* responded to injections of sufficiently strong extracts of muscle and gill, as well as to weak egg albumin solutions (Fig. 2). Such preparations, particularly the latter, certainly do not contain chromatophorotropins, and the melanophore response in such cases probably is part of a rather generalized stimulation resulting from the introduction of foreign substances into the hemolymph. Since it was essential to eliminate such responses when testing extracts of central nervous organs for chromatophorotropins, this was done by making up the extracts from comparable volumes of tissue, small enough so that extracts of them would not contain sufficient protein or other unknown non-humoral material to affect the chromatophores. In this way it was intended to distinguish specific chromatophorotropic effects from the non-specific effects resulting from injection of large tissue masses. In order to make up such extracts it was necessary to know the amounts of tissue in the different organs extracted. Since the organs, especially the sinus glands, were too small to weigh on an analytical balance, measurements were made of their volumes using a procedure suggested by the method of Weil and Pantin (1931) for measuring volume changes in the turbellarian, *Gunda ulvae.* The organ was carefully dissected out, placed in the ruled area of a hemacytometer counting chamber and flattened under the cover glass. An enlarged (1 mm. = 2 in.) outline of the organ was drawn on a piece of paper containing a copy of the ruled area of the hemacytometer; this was traced onto a piece of medium weight drawing paper, cut out, and weighed. By comparing this weight with that of a similarly enlarged square millimeter (0.1 cu. mm.) the volume of the organ could be roughly determined. Table I gives the results of these measurements. All crabs used had a carapace width of 1.7 cm. The numbers are the weights of the paper cutouts in milligrams.

These measurements are admittedly crude, most of the error being due to dissection. They do, however, give some idea of the relative size of the organs involved. The dilution factors are selected values, based on the relative volumes by which extracts of the different organs were diluted to give approximately equal volumes of tissue in the same amounts of extract. For organs other than those listed in Table I (brain, leg nerve, etc.) the volume was measured by the preceding method, and the extract was diluted accordingly.

In preparing extracts, the size of the crab from which the organ was extracted was considered. It was assumed that the size of the sinus gland and other organs varies directly with the weight of the crab; thus, for example, the sinus gland from a crab 1.9 cm. wide (3.0 g.) would be twice the volume of that from a crab 1.5 cm. wide (1.5 g.).

The amount of extract injected was always 5 per cent of the body weight, assuming a specific gravity of 1.00 for the extract. To obviate weighing each crab, a large number of crabs were weighed and the weight plotted against the

TABLE I

Comparative size of sinus gland and optic ganglia

	Sinus gland	Medulla terminalis	Medulla interna	Medulla externa	Lamina ganglionaris	1 mm.² paper
	19	607	308	311	207	377
	14	767	284	293	282	364
	16	501	272	341	250	355
	18	579	249	250	198	365
	12	550	236	259	157	376
	14	350	210	279		374
av.	15.5	559	260	289	219	369
$\frac{\text{av.}}{3690}$ = mm.³	0.004	0.151	0.070	0.078	0.059	0.100
Relative volume	1	36.1	16.8	18.7	14.17	
Dilution factor	1	30	15	15	12	

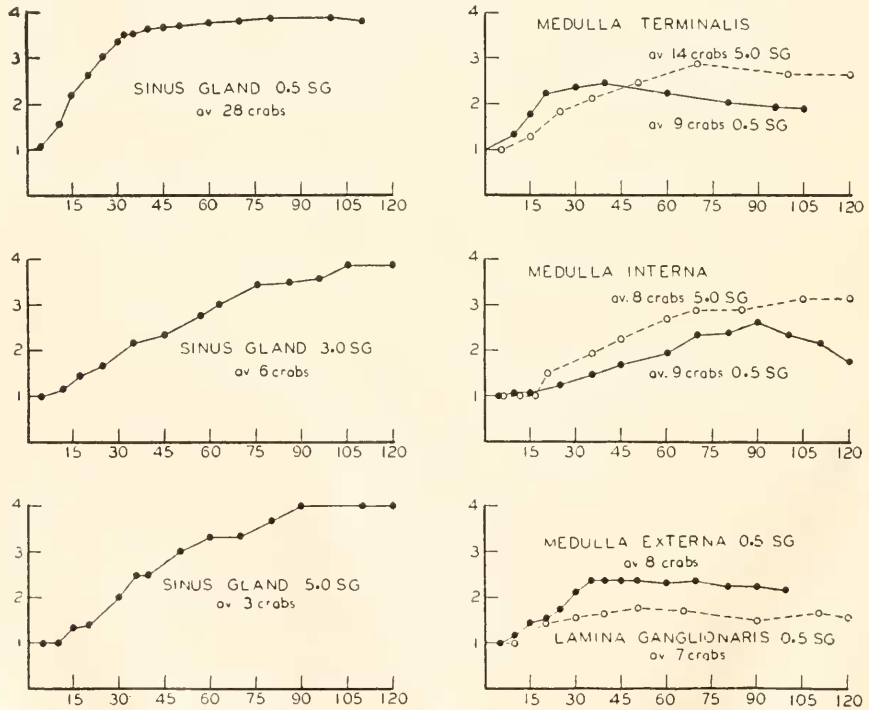

FIGURE 1. Responses of Hemigrapsus melanophores to injections of extracts of various organs. Abscissae: time (minutes) after injection. Ordinates: degree of dispersion of melanin (1 = complete concentration; 4 = complete dispersion).

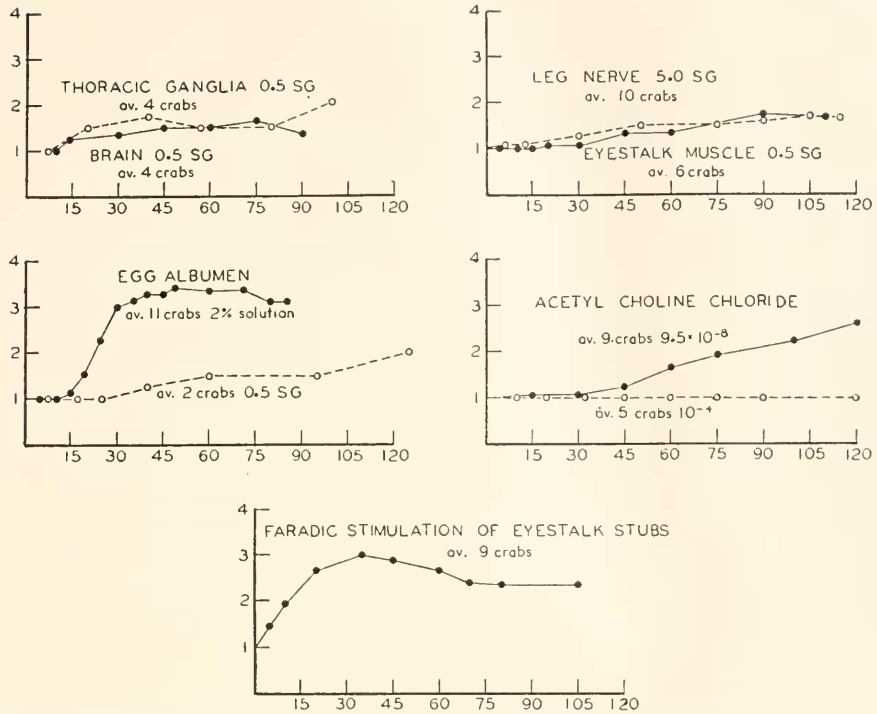

FIGURE 2. Responses of Hemigrapsus melanophores. Explanation same as for Figure 1.

carapace width. This made it possible simply to measure the carapace width and to inject the proper amount. Injections were made at the base of a walking leg.

The strength of each extract is expressed in terms of the volume of a sinus gland of the crab injected. Thus "0.5 SG" (cf. Fig. 1) indicates that a crab injected with 5 per cent of its body weight of this extract received a volume of tissue approximately equal to 0.5 times the volume of one of its own sinus glands.

EXPERIMENTS AND RESULTS

The results of the injections are shown in Table II and in the graphs of Figures 1 and 2. Responses are classified as "weak" when the melanin was not dispersed beyond stage 2, and "good" when stage 3 was reached. The average responses do not include those animals which failed to respond. The validity of averaging the arbitrary figures of the melanophore index is subject to criticism (Parker, 1948, pp. 14–15), and the variability of response, shown in Table II, must be considered when evaluating a response.

By far the most potent extracts were those of sinus glands. Other extracts from comparable volumes of tissue, while in some cases acting as rapidly as sinus gland extracts, did not produce the maximum and sustained responses which always followed injections of the latter. Moreover, the only extracts which always produced 100 per cent "good" responses were those of sinus glands. The more rapid

TABLE II

Summary of experiments

Organ extracted	Strength of extract (vol. of one of own sinus glands = 1)	No. of crabs injected	Responses		
			None	Weak	Good
Sinus gland	0.5 SG	28	0	0	28
Sinus gland	3.0 SG	6	0	0	6
Sinus gland	5.0 SG	3	0	0	3
Medulla terminalis	0.5 SG	11	2	4	5
Medulla terminalis	5.0 SG	15	1	5	9
Medulla interna	0.5 SG	13	4	5	4
Medulla interna	5.0 SG	8	0	1	7
Medulla externa	0.5 SG	8	0	5	3
Lamina ganglionaris	0.5 SG	17	10	5	2
Brain	0.5 SG	8	4	4	0
Thoracic ganglia	0.5 SG	12	8	3	1
Eyestalk muscle	0.5 SG	13	7	5	1
Leg nerve	5.0 SG	21	11	9	1
Egg albumin	0.5 SG	14	12	2	0
Egg albumin	2% sol'n	11	0	2	9
Acetylcholine	9.5×10^{-8}	26	17	5	4
Acetylcholine	10^{-4}	5	5	0	0
Faradic stimulation of eyestalk stubs		10	1	1	8

responses to the weaker (0.5 SG) extract are difficult to understand, but because of the small number of crabs injected with the stronger sinus gland extracts and the extent of individual variability in responsiveness, it is not possible to compare the responses adequately.

The responses to optic ganglia extracts with a concentration of 0.5 SG were in most cases weak, but these extracts probably did contain specific chromatophorotropins, since the responses to 0.5 SG egg albumin solutions were so slight, and the responses to eyestalk muscle extracts of the same concentration were also insignificant. However, the amounts of hormone in these optic ganglia extracts, especially those of lamina ganglionaris, appear to have been close to the threshold for the melanophores of *Hemigrapsus oregonensis*.

The responses to optic ganglia extracts of the concentration 5.0 SG were more definite, although they were much weaker than the responses to sinus gland extracts. It can be safely said, therefore, that the medulla terminalis and medulla interna (and probably the other optic ganglia) contain material that causes dispersion of the melanophores in *Hemigrapsus oregonensis*. The importance of this to the normal crab might be determined by removing the sinus glands without destroying the optic ganglia.

The possibility remained that the melanophores were responding to the acetylcholine present in the extracts. Welsh (1939) found large amounts of acetylcholine in leg nerves and ventral ganglia of Carcinides (= Carcinus), there being about five times as much in ganglia (about 10γ/g.) as in fibers (about 2γ/g.), while Smith (1939) found up to 20γ/g. in nerve fibers and up to 66γ/g. in

the ganglia of *Cambarus limosus*. It is improbable, however, that the chromatophorotropic effects of Hemigrapsus ganglia extracts are due to their acetylcholine content, since cholinesterase is probably also present in these extracts (Marnay and Nachmansohn, 1937). Moreover, Abramowitz and Abramowitz (1938) obtained slight responses of the chromatophores in only 20 per cent of the Uca they injected with acetylcholine. When injected with 5 per cent of their body weight of 9.5×10^{-8} acetylcholine chloride, each Hemigrapsus received the amount of acetylcholine that would have been present in tissue equal in volume to five times one of its own sinus glands, assuming 50γ/g. as the concentration of acetylcholine in this tissue. Seventeen out of twenty-six crabs thus injected failed to respond, and five crabs injected with the much stronger 10^{-4} acetylcholine also showed no response. This makes it fairly certain that the responses to optic ganglia extracts were not caused by the acetylcholine contained in them.

Responses to extracts of brain and thoracic ganglia of the concentration 0.5 SG were in most cases weak or absent, and they give little information as to whether or not chromatophorotropins are contained in these organs. A few injections of much stronger extracts of brain resulted in good responses, but these experiments were not well controlled. The good response to about ten seconds' faradic stimulation of one of the eyestalk stubs with a Harvard inductorium shows, however, that substances affecting the melanophores can be released in the eyestalkless Hemigrapsus, most probably from some part of the central nervous system. Deep probing of eyestalkless Hemigrapsus with a hypodermic needle at the base of the third or fourth leg also caused melanin dispersion in some cases. It is possible that the melanin dispersion following injections of muscle extracts and egg albumin solutions is an indirect response, caused by the release of chromatophorotropins from central nervous sources.

It is interesting to note that responses to leg nerve extracts were weak or absent, indicating that if a chromatophore hormone is present in nervous tissue it may be produced by or concentrated in the central nervous system rather than the peripheral nerves.

Discussion

It must be emphasized that these experiments do not compare the total amount of chromatophorotropic hormone available to the animal from one organ with that available from another organ, but indicate that while this hormone is most concentrated in the sinus gland it is not absent from certain parts of the central nervous system. It is entirely possible that in *Hemigrapsus oregonensis* as much or more hormone is present in central nervous system sources as in the sinus glands, although the present work does not provide quantitative information concerning this point. Although Brown (1940) found that 80 per cent of the chromatophorotropic material in the eyestalks of several species of shrimps and crabs was referable to the sinus gland, Smith (1948) has recently presented evidence that only about one-third of the retinal pigment activator in the eyestalks of *Hemigrapsus oregonensis* and two other species of grapsoid crabs resides in the sinus glands. It seems not unlikely that the distribution of the melanophore activator in the eyestalks of *Hemigrapsus oregonensis* is comparable.

The concentration of chromatophorotropins in the histologically specialized and

well innervated sinus gland may represent an adaptation for the storage and more especially the release of active substances in effective amounts and within short periods of time. Production of the active principles themselves might be by nervous tissues in general, or, as seems more likely, might be limited to more or less restricted regions of specialized cells within the central nervous system, including the sinus gland itself. Thus we could imagine that the chromatophorotropins in any given mass of nervous tissue are derived from a relatively few cells, each as specialized as sinus gland cells. These cells could be evenly distributed, resulting in a uniform distribution of hormone throughout the central nervous system, as Brown and Saigh (1946) found for CDH in the isopod, *Idothea baltica*. On the other hand, as in the case of CDH and CBLH in Crago, they could be restricted to a single organ (the tritocerebral commissure, Brown, 1946). The sinus gland, as Turner (1948, p. 561) points out, probably represents the highest evolutionary stage in the differentiation of endocrine tissue from the central nervous system in the Crustacea, and would therefore be expected to contain the highest concentrations of active materials. The experiments reported herein show this to be the case for chromatophorotropins in the sinus gland of *Hemigrapsus oregonensis*.

Summary

1. The melanophores of *Hemigrapsus oregonensis* become punctate after eyestalk removal.

2. Chromatophorotropins, which cause dispersion of the melanin when injected, are present in greatest concentration in the sinus gland, and are also present in the optic ganglia and possibly in the brain and thoracic mass of ganglia.

3. The melanin dispersion in response to electrical stimulation of the eyestalk stubs and to deep probing with a hypodermic needle indicates that some source of releasable chromatophorotropins exists other than the eyestalks.

4. The total amount of chromatophorotropins in the sinus gland is not necessarily greater than in any of the central nervous organs. The specialized structure and the innervation of the sinus gland suggests that its importance lies in its ability to store and rapidly release effective amounts of chromatophorotropins.

5. Injection of sufficient amounts of certain substances, including muscle and gill extracts and egg albumin solution, also induces melanin dispersion in eyestalkless *Hemigrapsus oregonensis*. It is suggested that these substances do not contain chromatophorotropic hormones, but the response to them is the result of a more general stimulation causing the release of chromatophorotropins from central nervous sources.

LITERATURE CITED

Abramowitz, A. A. and R. K. Abramowitz, 1938. On the specificity and related properties of the crustacean chromatophorotropic hormone. *Biol. Bull.*, **74**: 278–296.

Brown, F. A., Jr., 1940. The crustacean sinus gland and chromatophore activation. *Physiol. Zool.*, **13**: 343–355.

Brown, F. A., Jr., 1944. Hormones in the Crustacea: their sources and activities. *Quart. Rev. Biol.*, **19**: 32–46, 118–143.

Brown, F. A., Jr., 1946. The source and activity of Crago-darkening hormone (CDH). *Physiol. Zool.*, **19**: 215–223.

Brown, F. A., Jr. and L. M. Saigh, 1946. The comparative distribution of two chromatophorotropic hormones (CDH and CBLH) in crustacean nervous systems. *Biol. Bull.*, **91**: 170–180.

Marnay, A. and D. Nachmansohn, 1937. Cholinesterase dans le nerf de Homard. *C. R. Soc. Biol., Paris,* **125**: 1005.

Pantin, C. F. A., 1934. On the excitation of crustacean muscle. I. *Jour. Exp. Biol.*, **11**: 11–27.

Parker, G. H., 1948. Animal colour changes and their neurohumours. Cambridge University Press, Cambridge, England.

Smith, R. I., 1939. Acetylcholine in the nervous tissues and blood of crayfish. *Jour. Cell. Comp. Physiol.*, **13**: 335–344.

Smith, R. I., 1948. The role of the sinus gland in retinal pigment migration in grapsoid crabs. *Biol. Bull.*, **95**: 169–185.

Turner, C. D., 1948. General endocrinology. W. B. Saunders Co., Philadelphia.

Weil, E. and C. F. A. Pantin, 1931. The adaptation of Gunda ulvae to salinity. II. The water exchange. *Jour. Exp. Biol.*, **8**: 73–81.

Welsh, J. H., 1939. Chemical mediation in crustaceans. I. The occurrence of acetylcholine in nervous tissues and its action on the decapod heart. *Jour. Exp. Biol.*, **16**: 198–219.